Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Microbiology 114 (2006) 196–204
veterinary microbiology
www.elsevier.com/locate/vetmic

Multiple outbreaks of severe acute BVDV in North America occurring between 1993 and 1995 linked to the same BVDV2 strain

Julia F. Ridpath [a,*], John D. Neill [a], Stefan Vilcek [b], Edward J. Dubovi [c], Suzanne Carman [d]

[a] *Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, 2300 Dayton Avenue, P.O. Box 70, Ames, IA 50010, USA*
[b] *Department of Infectious Diseases, University of Veterinary Medicine, Komenskeho, Slovakia*
[c] *Molecular Diagnostic Laboratory, AHDL, Department of Population Medicine and Diagnostic Science, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA*
[d] *Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, Ont., Canada*

Abstract

The first reported outbreak of bovine viral diarrhea (BVD) in 1946 described a transmissible acute disease characterized by severe leukopenia, high fever, gastrointestinal erosions and hemorrhages. However, in the ensuing years, the most commonly observed acute form of BVD was clinically mild. There was limited viral shed and spread following these acute infections. This led to the assumptions that acute infections with BVD viruses (BVDV) were clinically unimportant, spread of the virus within a group was always due to the presence of a persistently infected animal and transmission between healthy immunocompetent cattle was insignificant. These assumptions were challenged when outbreaks of severe acute BVDV were observed in North America starting in the late 1980s. This study demonstrates that widespread outbreaks of severe acute BVD observed in 1993 in North America can be traced to a single strain of BVDV that apparently spread explosively following acute infection. These findings are notable in that they draw into question management of acute BVD infection, design of studies examining virulence and nomenclature used to identify strains for GenBank submission.
Published by Elsevier B.V.

Keywords: Bovine viral diarrhea virus; BVDV2; High virulence BVDV strains; Severe acute BVD

1. Introduction

When first described by Cornell University researchers in 1946 (Olafson et al., 1946) bovine viral diarrhea (BVD) was characterized as an acute

* Corresponding author. Tel.: +1 515 663 7586; fax: +1 515 663 7458.
E-mail address: jridpath@nadc.ars.usda.gov (J.F. Ridpath).

0378-1135/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetmic.2005.11.059

transmissible viral infection marked by severe leucopenia, high fever, depression, diarrhea, gastrointestinal erosions and hemorrhages. In that initial study of five herds, morbidity was 33–88% and mortality was 4–8%. Subsequently, Pritchard (1963) identified four forms of infection associated with the BVD virus (BVDV); BVD-mucosal disease (BVDV-MD), chronic BVD (now recognized as a form of BVD-MD), mild acute BVD and severe acute BVD. While the first report of BVD in the literature was the severe acute form, acute BVD infections came to be regarded as clinically unimportant and textbooks of the day stated that the transmission of the virus between healthy immunocompetent cattle was probably insignificant (Radostits et al., 1994). In 1987, Perdrizet et al. (1987) reported thrombocytopenia with hemorrhage associated with BVD virus infection occurring in dairy herds in the northeastern United States. Corapi et al. (1989) isolated a virus, CD 87, from an outbreak of severe acute BVD in New York state. In this outbreak, morbidity and mortality were 50 and 20%, respectively, with clinical signs including high fever, bloody diarrhea, hemorrhages and prolonged bleeding from venepucture sites. This disease came to be regarded as a distinct form of severe acute BVD termed hemorrhagic syndrome. Corapi et al. (1989) were able to experimentally reproduce hemorrhagic syndrome using the CD 87 virus. In 1992, Bolin and Ridpath (1992) identified a second isolate, 890, that could cause severe acute BVD under experimental conditions. Severe acute BVD cases were reported with increasing frequency in North America in the early 1990s. These outbreaks were particularly devastating in the Canadian provinces of Quebec and Ontario. In the province of Quebec, a BVD epidemic starting in early 1993 resulted in the death of 32,000 out of 143,000 (22.4%) animals in the 1993 veal calf crop (Pellerin et al., 1994). A virus, called IAF 103, was isolated from the Quebec epidemic. In Ontario outbreaks of severe acute BVDV, also occurring in 1993, affected an estimated 150 dairy, 660 beef and 100 veal calf herds with death seen in all age groups and economic losses reaching $100,000 in severely affected herds (Carman et al., 1998). Two viruses, called 1373 and 24515, were isolated from animals housed on two different farms involved in the Ontario epidemic. Concurrently, a BVDV strain called NY 93 was isolated in 1993 from an outbreak of severe acute BVD observed in one dairy herd following the importation of a heifer from Canada. All the viruses isolated from outbreaks of severe acute BVDV in North America were determined to belong to the BVDV2 genotype (Pellerin et al., 1994; Ridpath et al., 1994).

In this study, we compare the sequences of virulent viruses isolated before, during and after the 1993 North American epidemic of severe acute BVD to see if there are relationships between these viruses. We also compared the virulence of these viruses based on clinical signs following infection of seronegative, BVDV negative colostrum deprived calves.

2. Materials and methods

2.1. Viral isolates

Viruses used in these studies are listed in Table 1. All viruses were propagated as described previously with the exception that Madin Darby bovine kidney (MDBK) cell line was used rather than bovine turbinate cells (Ridpath et al., 2002). MDBK cells were grown in McCoy's cell culture medium supplemented with fetal bovine serum tested free of BVDV and antibodies against BVDV.

2.2. Preparation of template, polymerase chain reaction amplification, sequencing and analysis

To prepare RNA template, subconfluent monolayers of MDBK cells were inoculated with BVDV strains at an approximate multiplicity of infection of 10. Cultures, including cells and culture media, were harvested by two cycles of freezing and thawing at 20 °C 5 days post-inoculation. A 140 μl aliquot of the resulting lysate was used to prepare RNA using a QIAamp Viral RNA Mini Kit (Qiagen Sciences, Valencia, CA) per the manufacturer directions. The final volume of RNA template solution was 100 μl. A 10 μl aliquot of this solution was used per RT-PCR reaction performed as described previously (Ridpath and Bolin, 1998). Complete viral sequences were derived by direct sequencing of RT-PCR amplification products. Amplicons averaged 500 base pairs and successive amplicons overlapped by approximately 200 bases. All sequencing reactions were done in

Table 1
BVDV isolates

Viral isolate	Geographic origin	Year of isolation	Laboratory	Clinical presentation	1373 Insertion
IAF 103	Quebec	1993*	Institut Armand-Frappier	Severe acute BVD*	Yes
1373	Ontario	1993	University of Guelph	Pneumonia and scours, eventually lost 50 cows and 25 heifers	Yes
24515	Ontario	1993	University of Guelph	Aborted fetus	Yes
NY93	New York	1993	Cornell University	Severe acute BVD, death loss	Yes
690393	New York	1993	Cornell University	Respiratory disease, temperature 104–108 °C	No
CD 87	New York	1988	Cornell University	Hemorrhagic syndrome	No
890	Iowa	1990	NADC	Severe acute BVD, death loss	No
SDSU 37621a	Iowa	1993	NADC	Hemorrhagic syndrome, 30 deaths in 80 calves	Yes
793	Indiana	1993	NADC	Severe acute BVD	Yes

* Was not able to confirm information with Institut Armand-Frappier.

duplicate and all sequences were confirmed by sequencing both strands. The sequence of PCR primers used to survey the NADC BVDV collection and the region of the genome amplified are shown in Table 2. PCR products were quantitated using the Pico Green assay for dsDNA (Invitrogen Corporation, Carlsbad, CA). The appropriate quantity of dsDNA PCR product was labeled in both directions using Big Dye terminator chemistries (Applied Biosystems Inc., Foster City, CA) according to manufacturer's instructions. The labeled products were sequenced using an ABI 3100 genetic analyzer (Applied Biosystems Inc.). Primers used for sequencing were identical to those used in the primary PCR reaction shown in Table 2. Sequences were aligned and compared based on Higgins-Sharp algorithm (CLUSTAL4) using the MacDNASIS program (Hitachi Software, San Cruno, CA). This program takes as input a dendrogram produced by applying the unweighted pair group method using arithmetic average (UPGMA) (Sneath and Sokal, 1962) to a matrix of similarity score for all the aligned sequences. The similarity score are calculated as the number of exactly matched residues in a Wilbur and Lipman (1983) alignment minus a fixed penalty for every gap.

2.3. In vivo studies

Two- to 4-month-old mixed breed calves tested free of BVDV, by virus isolation, done out of buffy coat samples and free of BVDV antibodies in serum were used. Calves were housed individually in climate-controlled pens. They were inoculated via the nasal

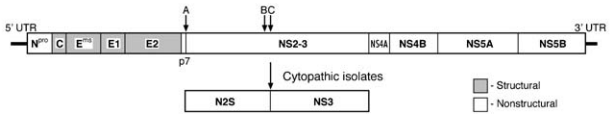
Table 2
Primers used to generate sequence information

Primer set	Sequence	Corresponding nucleotide position in SD-1[a]
5′ UTR primers		
Forward primer	5′-CAT GCC CAT AGT AGG AC-3′	108–125
Reverse primer	5′-CCA TGT GCC ATG TAC AG-3′	372–389
E2 primers		
Forward primer	5′-GGC CAT ATG AGA CGA CGA CA-3′	2261–2281
Reverse primer	5′-GGC GAC CAC TGC TAT GAT TA-3′	3502–3522
NS2/3 primers		
Forward primer	5′-TGC CAA GTT GGA GAC CTC TA-3′	4127–4147
Reverse primer	5′-CCT GAG GCA GTG ACA CA-3′	5585–5601

[a] Genomic location based on published sequence of the noncytopathic, low virulence, BVDV1a strain SD-1.

```
          1510        1520        1530        1540        1550        1560        1570
1373 PKCGRQGKPI TCGMTLADFE EKHYKKKFIR E**ESSCPVPFD PSCHCNYFRH D**GPFRKEYKG YVQYTARGQL
SD-1 PKCGRHGKPI TCGMSLADFE ERHYKRIFIR EGNFE----- ---------- -GPFRQEYNG FIQYTARGQL
```

(a)



(b)

Fig. 1. (a) Insertion in 1373. Inserted sequences detected in the highly virulent BVDV type 2 virus 1373. The 1373 ORF is 48 amino acids longer than the ORF of the noncytopathic BVDV reference strain SD-1. The difference in length of the two ORF's is due to a 16 amino acid insertion shown in bold print. This sequence is preceded by a four amino acid mismatch consisting of the sequence ESSC. The location of the insertion was at amino acid position 1545. (b) Reported insertions in cytopathic and noncytopathic BVDV. Arrow labeled A indicates position of insertion reported in the noncytopathic highly virulent BVDV 2 strain 890. The location of the insertion is at amino acid position 1161. Arrows labeled B and C indicate positions of insertions found in cytopathic BVDV. These arrows correspond to amino acid positions 1545 and 1589. Insertions at these locations in noncytopathic BVDV are correlated with cleavage of the NS2/3 into NS2 and NS3. The insertion identified in 1373 is at position B (amino acid residue1545).

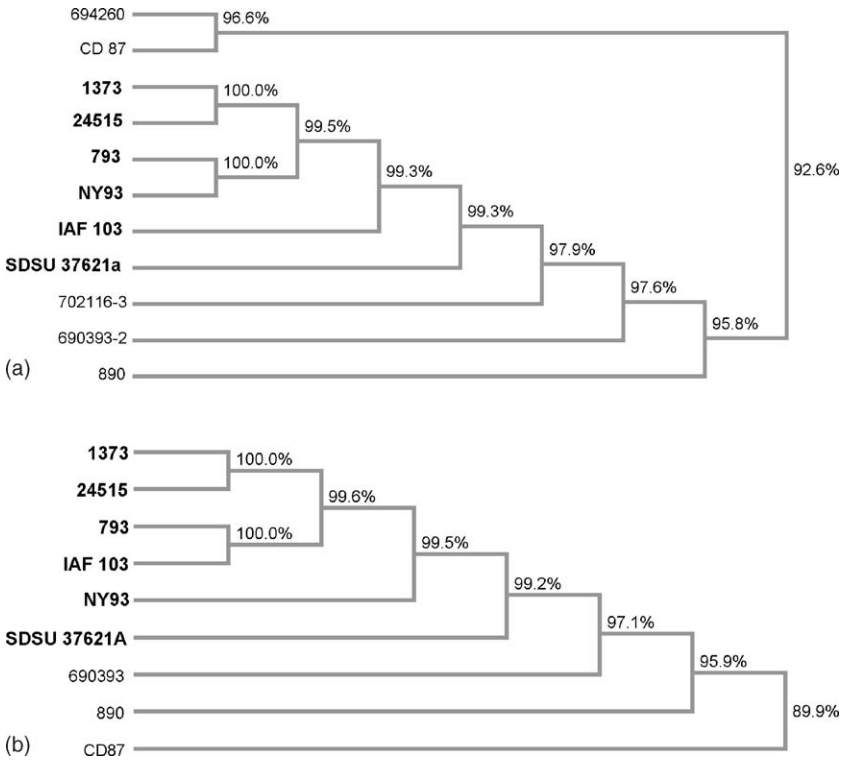


Fig. 2. (a) Phylogenetic analysis of 5′ UTR nucleotide sequences. Sequences derived from the 5′ UTR from the five viruses containing the insertion (1373, 24515, 793, NY93, IAF 103 and SDSU 37621a) were compared to type 2 BVDV that were either isolated in the same time frame and region as NY 93 (isolates 694260, 702116-3, 690393-2) or were associated with severe acute BVD outbreaks (CD 87, 890), (b) Phylogenetic analysis of predicted amino acid sequence from E2 region. The amino acid sequences predicted from nucleotide coding sequences derived from the E2 region were compared. The predicted amino acid sequences from the five viruses that contained the insertion (1373, 24515, 793, NY93, IAF 103 and SDSU 37621a) were compared to type 2 BVDV that were either isolated in the same time frame and region as NY 93 (690393-2) or were associated with severe acute BVD outbreaks (CD 87, 890).

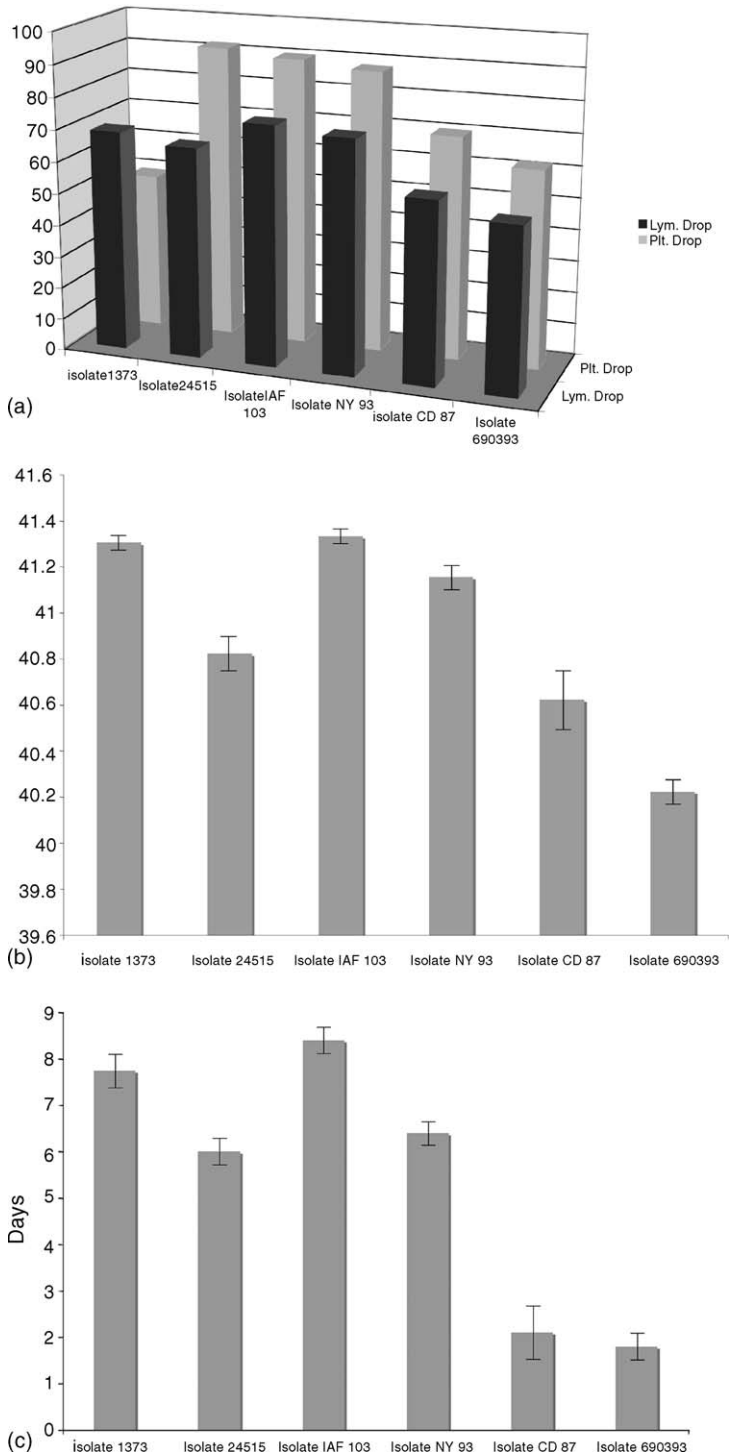


Fig. 3. (a) Changes in circulating lymphocyte and platelet counts following infection. The average of the percent decrease in circulating lymphocyte and platelet numbers observed following infection with each isolate is shown. The percent decrease was derived by taking the lowest

Table 3
Viral isolation from buffy coat samples

Strain	# Animals infected	% positive by VI from buffy coat					
		Baseline	Day 3	Day 6	Day 9	Day 11	Day 13
1373	6	0	100	100	100	100	50
24515	10	0	50	100	90	50	40
IAF 103	5	0	100	100	100	80	40
NY 93	5	0	100	100	100	80	40
CD 87	5	0	100	100	100	40	40
690393	5	0	100	100	100	0	0
Control (noninfected[a])	# Animals						
	7	0	0	0	0	0	0

[a] At least one control animal (noninfected), housed in same barn but not in contact with infected animals, was included in each experimental group.

route with 5 ml of freeze thaw lysate, prepared from infected MDBK cells 5 days after inoculation, that contained approximately 1×10^6 tissue culture infectious doses (TCID) of virus per ml. Temperatures were taken daily and blood samples were collected pre inoculation and on days 3, 6, 9, 11 and 13 days post-inoculation. Lymphocyte and platelet counts were determined using a cytometer per the manufacturer's directions (CDC Technologies, Inc., Oxford, CT). Number of animals inoculated with each virus is shown in Table 3.

3. Results

Isolate 1373 is a noncytopathic BVDV2. The genome of the 1373 strain is 12333 bp long (GenBank accession AF145967). The open reading frame (ORF) extends from nucleotide position 389–12,128. The 1373 ORF is 48 nucleotides longer than the ORF of the noncytopathic BVDV reference strain SD-1 (Deng and Brock, 1992). The difference in length of the two ORF's is due to a 16 amino acid insertion (Fig. 1a) with the following sequence: PVPFDPSCHCNYF RHD. This sequence is preceded by a four amino acid mismatch consisting of the sequence ESSC. The location of the insertion was at amino acid position 1545. Insertions in cytopathic BVDV2 are seen in this area (Fig. 1b). A blast search using the sequence ESSCPVPFDPSCHCNYFRHD showed that this sequence was only found in two other GenBank accessions, AF502399.1 and AY149216.1. These two accessions correspond to the genomic sequences for the BVDV isolates NY 93 and 24515. A Blast search conducted using the complete genomic sequence of 1373 revealed that there was a greater than 99% sequence identity between 1373 and these two isolates. A survey of the NADC collection of BVDV isolates identified three other isolates, IAF 103, SDSU 37621a and 793 that contained the insertion. We did not find the insertion in the isolates CD 87 or 890, which had previously been reported as causative agents in severe acute BVD or in isolates procured from the Cornell BVDV collection that were isolated in the same time frame and region as NY93. Sequences derived from the 5′ UTR from six viruses containing the insertion (1373, 24515, 793, NY93, IAF 103 and SDSU 37621a) were compared to type 2 BVDV that were either isolated in the same time frame and region as NY 93 (isolates 694260, 702116-3, 690393-2) or were associated with severe acute BVD outbreaks (CD 87, 890). The 5′ UTR sequences from the six viruses with the insertion had greater than 99% nucleotide sequence identity (Fig. 2a). In contrast, sequence identity between these six viruses and the other viruses in the analysis ranged from 97.9 to

lymphocyte (lym) or platelet (plt) count observed for each animal, dividing by the lymphocyte or platelet count observed in that animal before infection, and then multiplying by 100 to give percentage. Error bars represent standard error of the mean. (b) The highest temperature observed for each animal in the 14 days following infection were averaged per group. Temperatures values are shown on the vertical axis. Isolates are listed on the horizontal axis. Error bars represent standard error of the mean. (c) Duration of fever following infection with BVDV isolates. Average number of days in which the basal temperature exceeded 40 °C is shown for each isolate. Error bars represent standard error of the mean.

92.6%. Similarly, a greater than 99% amino acid sequence identity in the E2 coding region was observed between five viruses with the insertion while sequence identities between these isolates and BVDV isolates that did not contain the insertion ranged from 89.9 to 97.1% (Fig. 2b).

Virus was isolated from the buffy coat of all inoculated animals on at least one sampling date between days 3 and 13 post-inoculation. This indicates that all animals developed viremia during the course of infection. The length of viremia was shorter in animals inoculated with 690393 compared to animals inoculated with the other six viruses. Infection of colostrum deprived calves with 1373, 24515, NY 93, IAF 103, CD 87 or 690393 all resulted in the development of clinically severe disease characterized by febrile response, leukopenia and thrombocytopenia (Fig. 3a and b). Clinical outcome of infections were similar to those seen in previous studies in our laboratory using the 890 isolate (Bolin and Ridpath, 1992). On the basis of fever exceeding 40 °C, lymphocyte drops approaching 50% and platelet drops exceeding 40% these six viruses would be categorized as high virulence. One animal in each of the groups infected with 1373, 24515, IAF 103 and CD 87 became moribund and was euthanized in the first 14 days following infection. Higher temperatures and a longer duration of fever were observed in animals infected with 1373, 24515, NY 93 and IAF 103 than in animals infected with CD 87 or 690393 (Fig. 3c). The implications of these differences are unclear. There was not a consistent correlation between febrile response and platelet drop or animals becoming moribund as evidenced by animals inoculated with CD 87. Regardless of the observed differences these data clearly demonstrate that all animals developed viremia and clinically severe disease following inoculation.

4. Conclusions

Analysis of the first published sequence for a high virulence noncytopathic virus, 890, revealed a 228 nucleotide (76 amino acid) insertion preceding the amino terminus of the NS2/3 nonstructural protein (Ridpath and Bolin, 1995b). Within this 76 amino acid insertion was a 73 amino acid sequence that was a duplication of amino acid residues 1087–1160. The insertion found in 890 was the first in a noncytopathic virus and the first observed in this location. Previously insertions had been reported in a number of cytopathic viruses (Meyers et al., 1991a,b, 1992, 1998; Neill and Ridpath, 2001; Qi et al., 1992, 1998; Ridpath and Bolin, 1995a; Ridpath and Neill, 2000; Rinck et al., 2001; Tautz et al., 1993, 1996; Vilcek et al., 2000).

These insertions occurred within the region coding for the NS2/3. Five other BVDV strains isolated from cattle that had died of severe acute BVD were surveyed to see if the insertion was characteristic of high virulence BVDV noncytopathic strains (Ridpath and Bolin, 1995b). This insertion was not detected in the five strains tested. The detection of the insertion in 1373, while different in size, content and location, once again raised the question of whether insertions in noncytopathic BVDV play a role in virulence. Our interest was piqued when this same insertion was found in published sequences of two other BVDV isolates associated with severe acute BVD. The finding of this sequence in three additional viruses associated with severe acute BVD, contained in the NADC collection, suggested that this insertion could well be a marker for virulence. However, further analysis revealed that all strains possessing the insert had sequence identities above 99%. This indicated that rather than observing a marker common to several strains of BVDV, we were observing the reisolation of one BVDV strain in several locations.

These findings are notable in that they draw into question management of acute BVD infection, design of studies examining virulence and nomenclature. Conventional wisdom holds that BVD outbreaks are subclinical in healthy animals, can nearly always be traced to contact with a persistently infected animal and that transmission of the virus following acute infection is insignificant to the spread of virus. Thus, the observation that several different outbreaks of severe acute BVD observed in North America were due to a single strain of BVDV that spread explosively following acute infection requires a paradigm shift in our concept of BVDV transmission. The spread of low virulence strains within a population is most likely the result of contact with a persistently infected (PI) animal, and thus the number of contacts available to the PI animal limits spread. The spread of highly virulent BVDV strains within cattle populations is

more similar to that of classical swine fever viruses. Transmission by acutely infected animals is significant and the sources of virus increase as the number of infected animals increases. Thus, management to control outbreaks of severe acute BVD requires a different approach than that used for subclinical BVD. Clear criteria that differentiate strains that cause severe acute BVD from less virulent strains would aid in the development of these management strategies. From this and previous studies (Bolin and Ridpath, 1992; Corapi et al., 1989), it appears that three clinical presentations accompany severe acute BVD. They are pronounced and prolonged febrile response (>104 F, lasting for >3 days), pronounced reduction in circulating white blood cells (>40%) and pronounced reduction in platelets (>40%).

However, it should be stressed that not all high virulence viruses are related. A previously published study (Topliff and Kelling, 1998) proposed that different sequence motifs located in the 5′ UTR of type 2 BVDV could be used to predict the virulence level of viruses. While the sequences of four low virulence viruses and four high virulence viruses were used in this analysis, the authors did not consider the similarity of the viruses used or the possibility of common origins. That is, genetically divergent viruses were not used. While the authors show similar motifs among viruses with similar virulence levels they did not determine if this were due to the existence of a virulence marker or due to the fact that they happened to select a group of viruses that were similar. The proposed prediction scheme labels CD 87 a low virulence virus, suggesting that the latter rather than the former assumption is correct. In this same vein, failure to recognize that 1373, 24515, IAF 103 and NY-93 are all the same strain could lead to misinterpretation of results. There are virulent viruses, such as 890 and CD 87 that are not related to 1373. When searching for commonalities among virulent strains that may be used as virulence markers it is important to compare divergent rather than closely related strains.

This leads us to the question of the terminology that should be used in referring to the strain of virus represented by 1373, 24515, IAF 103 and NY-93. There are already three GenBank submissions of isolates of this strain labeled as 1373, 24515 and NY-93. A researcher not familiar with the history of these isolates might assume that they represent three different strains leading to faulty design premises. Similarly research, such as pathogenesis studies, might be unnecessarily repeated if it is not understood that these are all isolates of the same strain. On the other hand, noting the reisolation of this strain in different location leads us to a better understanding of the epidemiology of severe acute BVD. One solution would be to denote viruses with complete sequence identities of greater than 99% to this strain and possessing the insertion identified in this study as SA 93 (Severe acute 1993, referring to clinical presentation and starting year of the North American epidemic) followed by the isolate name. Thus, 1373 would be SA 93 1373 and 24515 would be SA 93 24515. This leads to the broader question of nomenclature for BVDV strains in general. How different must two isolates be in order to be considered two different strains? This is more than an esoteric taxonomy question. BVDV are variable by nature. In propagating a vaccine strain some mutations may accumulate with passage. What level of variation is necessary before regulatory agencies should consider new licensure qualifications? On occasion, biologics companies will reisolate or clone strains to be used in vaccines from previously isolated vaccine or laboratory strains, and then give these strains new names. Is this practice acceptable from a taxonomic standpoint?

These results suggest that other high virulence viruses were present in North America prior to 1993, as represented by CD 87 and 890. However, it appears that outbreaks of severe acute BVD in the Canadian provinces of Quebec and Ontario and the U.S. states of New York, Iowa and Indiana were due to the explosive spread of one BVDV strain. The question of the origin of this virus remains. The epidemic was over by 1995 and this strain apparently has not reappeared in the field. Studies are now underway to determine if the observed insertion may have contributed to the virulence and/or spread of this virus.

Acknowledgements

The authors thank the following individuals for their contributions to this manuscript; Margaret Walker (technical support), Bruce Grey (animal care),

Mike Marti (illustrations), Harold Ridpath (statistics) and Sue Ohlendorf (manuscript preparation). Their help was invaluable and much appreciated.

References

Bolin, S.R., Ridpath, J.F., 1992. Differences in virulence between two noncytopathic bovine viral diarrhea viruses in calves. Am. J. Vet. Res. 53 (11), 2157–2163.

Carman, S., van Dreumel, T., Ridpath, J., Hazlett, M., Alves, D., Dubovi, E., Tremblay, R., Bolin, S., Godkin, A., Anderson, N., 1998. Severe acute bovine viral diarrhea in Ontario, 1993–1995. J. Vet. Diagn. Invest. 10 (1), 27–35.

Corapi, W.V., French, T.W., Dubovi, E.J., 1989. Severe thrombocytopenia in young calves experimentally infected with noncytopathic bovine viral diarrhea virus. J. Virol. 63 (9), 3934–3943.

Deng, R., Brock, K.V., 1992. Molecular cloning and nucleotide sequence of a pestivirus genome, noncytopathic bovine viral diarrhea virus strain SD-1. Virology 191 (2), 867–869.

Meyers, G., Rumenapf, T., Tautz, N., Dubovi, E.J., Thiel, H.J., 1991a. Insertion of cellular sequences in the genome of bovine viral diarrhea virus. Arch. Virol. Suppl. 3, 133–142.

Meyers, G., Stoll, D., Gunn, M., 1998. Insertion of a sequence encoding light chain 3 of microtubule-associated proteins 1A and 1B in a pestivirus genome: connection with virus cytopathogenicity and induction of lethal disease in cattle. J. Virol. 72 (5), 4139–4148.

Meyers, G., Tautz, N., Dubovi, E.J., Thiel, H.J., 1991b. Viral cytopathogenicity correlated with integration of ubiquitin-coding sequences. Virology 180 (2), 602–616.

Meyers, G., Tautz, N., Stark, R., Brownlie, J., Dubovi, E.J., Collett, M.S., Thiel, H.J., 1992. Rearrangement of viral sequences in cytopathogenic pestiviruses. Virology 191 (1), 368–386.

Neill, J.D., Ridpath, J.F., 2001. Recombination with a cellular mRNA encoding a novel DnaJ protein results in biotype conversion in genotype 2 bovine viral diarrhea viruses. Virus Res. 79 (1–2), 59–69.

Olafson, P., McCallum, A., Fox, F., 1946. An apparently new transmissible disease of cattle. Cornell Vet. (36), 205–213.

Pellerin, C., van den Hurk, J., Lecomte, J., Tussen, P., 1994. Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. Virology 203 (2), 260–268.

Perdrizet, J.A., Rebhun, W.C., Dubovi, E.J., Donis, R.O., 1987. Bovine virus diarrhea–clinical syndromes in dairy herds. Cornell Vet. 77 (1), 46–74.

Pritchard, W.R., 1963. The bovine viral diarrhea-mucosal disease complex. Adv. Vet. Sci. Comp. Med. 41, 1–47.

Qi, F., Ridpath, J.F., Berry, E.S., 1998. Insertion of a bovine SMT3B gene in NS4B and duplication of NS3 in a bovine viral diarrhea virus genome correlate with the cytopathogenicity of the virus. Virus Res. 57 (1), 1–9.

Qi, F., Ridpath, J.F., Lewis, T., Bolin, S.R., Berry, E.S., 1992. Analysis of the bovine viral diarrhea virus genome for possible cellular insertions. Virology 189 (1), 285–292.

Radostits, O.M., Blood, D.C., Gay, C.C., 1994. Diseases caused by viruses and chlamydia. I. In: Veterinary Medicine, seventh ed. Bailliere Tindall, London, pp. 929–1033.

Ridpath, J.F., Bolin, S.R., 1995a. Delayed onset postvaccinal mucosal disease as a result of genetic recombination between genotype 1 and genotype 2 BVDV. Virology 212 (1), 259–262.

Ridpath, J.F., Bolin, S.R., 1995b. The genomic sequence of a virulent bovine viral diarrhea virus (BVDV) from the type 2 genotype: detection of a large genomic insertion in a noncytopathic BVDV. Virology 212 (1), 39–46.

Ridpath, J.F., Bolin, S.R., 1998. Differentiation of types 1a, 1b and 2 bovine viral diarrhoea virus (BVDV) by PCR. Mol. Cell Probes 12 (2), 101–106.

Ridpath, J.F., Bolin, S.R., Dubovi, E.J., 1994. Segregation of bovine viral diarrhea virus into genotypes. Virology 205 (1), 66–74.

Ridpath, J.F., Hietala, S.K., Sorden, S., Neill, J.D., 2002. Evaluation of the reverse transcription-polymerase chain reaction/probe test of serum samples and immunohistochemistry of skin sections for detection of acute bovine viral diarrhea infections. J. Vet. Diagn. Invest. 14 (4), 303–307.

Ridpath, J.F., Neill, J.D., 2000. Detection and characterization of genetic recombination in cytopathic type 2 bovine viral diarrhea viruses. J. Virol. 74 (18), 8771–8774.

Rinck, G., Birghan, C., Harada, T., Meyers, G., Thiel, H.J., Tautz, N., 2001. A cellular J-domain protein modulates polyprotein processing and cytopathogenicity of a pestivirus. J. Virol. 75 (19), 9470–9482.

Sneath, P.H., Sokal, R.R., 1962. Numerical taxonomy. Nature 193, 855–860.

Tautz, N., Meyers, G., Stark, R., Dubovi, E.J., Thiel, H.J., 1996. Cytopathogenicity of a pestivirus correlates with a 27-nucleotide insertion. J. Virol. 70 (11), 7851–7858.

Tautz, N., Meyers, G., Thiel, H.J., 1993. Processing of polyubiquitin in the polyprotein of an RNA virus. Virology 197 (1), 74–85.

Topliff, C.L., Kelling, C.L., 1998. Virulence markers in the 5′ untranslated region of genotype 2 bovine viral diarrhea virus isolates. Virology 250 (1), 164–172.

Vilcek, S., Greiser-Wilke, I., Nettleton, P., Paton, D.J., 2000. Cellular insertions in the NS2-3 genome region of cytopathic bovine viral diarrhoea virus (BVDV) isolates. Vet. Microbiol. 77 (1–2), 129–136.

Wilbur, W.J., Lipman, D.J., 1983. Rapid similarity searches of nucleic acid and protein data banks. Proc. Natl. Acad. U.S.A. 80 (3), 726–730.